

COPULATION BY HYPODERMIC INJECTION IN THE
NUDIBRANCHS *PALIO ZOSTERAE* AND *P. DUBIA*
(GASTROPODA, OPISTHOBRANCHIA)

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ABSTRACT

The Pacific nudibranch *Palio* (= *Polycera*) *zosteræ* and the Atlantic *P. dubia* copulate in a manner different from that of most nudibranchs. Nudibranchs are hermaphroditic and normally copulate reciprocally, with the intromittant organ of one inserted in the vaginal opening of the other. *P. zosteræ* and *P. dubia* lack a complete vaginal duct. Copulation is usually reciprocal but involves the piercing of the body wall by a barbed, eversible penial cirrus. If the cirrus injects exogenous sperm into the body wall or the haemocoel, the sperm are phagocytosed by blood cells. If the cirrus penetrates the gonad, exogenous sperm may be injected into a spermatogenic acinus. After traveling down the hermaphroditic duct to the anterior reproductive organs, these sperm presumably are sorted from endogenous sperm and stored in the receptaculum seminis. The organization of the reproductive system in *Palio* differs markedly from that in members of the closely related genus *Polycera*.

INTRODUCTION

Nudibranchs are hermaphroditic gastropod molluscs with a complex reproductive system (Lloyd, 1952; Pruvot-Fol, 1960; Ghiselin, 1966; Hyman, 1967; Schmekel, 1971). The gonad lies in the posterior of the animal and is connected via a hermaphroditic duct to the anterior reproductive organs. These lie just inside the gonopore in the right anterior quadrant of the animal. In the anterior organs the exogenous and endogenous sperm are stored in separate structures, and prostatic secretions are produced and mixed with endogenous sperm during copulation. Eggs are fertilized there and surrounded by albumen, a capsule, and the mucous stroma of the egg mass during oviposition.

Even though nudibranchiate reproductive systems vary greatly in their organization (see review by Ghiselin, 1966), adults typically reciprocally copulate with their gonopores aligned so that the penis of one deposits sperm in the vagina of the other (Costello, 1938; Hyman, 1967). The exogenous sperm received during copulation may be initially stored in the bursa copulatrix, but is eventually stored in the receptaculum seminis for later use in fertilizing the animal's eggs (Thompson, 1961; Ghiselin, 1966). At all times the sperm are confined within the reproductive system of the donor or the recipient.

This paper presents observations on a novel mode of copulation in the phanerobranch nudibranchs *Palio* (= *Polycera*) *zosteræ* (O'Donoghue, 1924) and *P. dubia* (Sars, 1824). These limaciform animals lack a complete vagina and copulate by piercing the body wall with a barbed penial cirrus. The fate of the injected sperm depends on where they are deposited within the recipient's body.

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MATERIALS AND METHODS

Palio zosterae were collected during summer months in the shallow subtidal zone in Mitchell Bay, San Juan Island, Washington. The nudibranchs typically were found feeding on the bryozoan *Membranipora membranacea* (Linnaeus, 1767) which grows on the kelp *Laminaria* sp. Nudibranchs were maintained in sea tables at the Friday Harbor Laboratories, Friday Harbor, Washington, where their copulatory behavior was observed.

Specimens for histological examination were relaxed with isotonic MgCl_2 , fixed in Bouin's, Hollande-Bouin's, or Zenker's fixative, embedded in paraffin, and serially sectioned at four to eight microns. Mounted deparaffinized sections were stained with either haematoxylin and eosin, Masson's trichrome, or Mallory-Heidenhain azon. To determine the fate of injected sperm, fifteen specimens were fixed without MgCl_2 treatment either during copulation, within one hour, or after 4, 8, 12, or 24 hours. These specimens were then serially sectioned and stained as described above.

Ten immature specimens were selected from collected nudibranchs. They were deemed immature due to their small size relative to adults (≤ 3 mm versus ≥ 7 mm), the lack of externally visible ovotestes, and their lack of copulatory and egg laying behavior. Eight of these were isolated in dishes of sea water and fed *Membranipora*. The remaining two were maintained in one dish as controls. In four weeks all ten were considered sexually mature because of their adult size, externally visible ovotestes, and the copulatory and egg-laying behavior of the control animals. Two of the isolated nudibranchs were fixed without having been allowed to copulate and then prepared for serial sectioning. The others were paired and their copulatory behavior observed. Six were then fixed at various times up to 24 hours after copulation, embedded in paraffin and serially sectioned. Two were kept together for an additional two days to see if they laid eggs.

For comparative purposes, *Palio dubia* and three species from the closely related genus *Polycera* were examined. *Palio dubia* were collected subtidally on pier pilings where they were feeding on *Bowerbankia gracilis* Leidy, 1855, at Gerrish Island, Kittery, Maine, and in the nearby rocky intertidal zone. These nudibranchs were maintained at the University of New Hampshire, Durham, New Hampshire, where their copulatory behavior was observed. Four specimens were fixed and serially sectioned while others were dissected. Preserved specimens of the Pacific *Polycera tricolor* Robilliard, 1971, and the European *P. faeroensis* Lemche, 1929, and *P. quadralineata* (Müller, 1776) were obtained for dissection and histological examination. Radulae and jaws of all species were examined for taxonomic purposes.

RESULTS

Copulatory behavior

Copulation in *Palio zosterae* involves the penetration of the partner's body wall by a penial cirrus. Two nudibranchs become sexually aroused upon contacting each other, and usually align head-to-tail with right sides opposing. A translucent preputium everts through the external gonopore located anteriorly on each animals' right side (Figs. 1, 2). The vas deferens runs from the animal's interior to the most distal point of the preputium. The cirrus is housed within the vas deferens. Just posterior to the vas deferens is a vaginal in-pocketing that ends blindly within the expanded preputium.

The distal end of the preputium is normally pressed against the side of the partner before the cirrus is everted. As the cirrus everts, proximally directed, serially arranged barbs covering the external surface of the cirrus are exposed (Fig. 3). The barbs appear

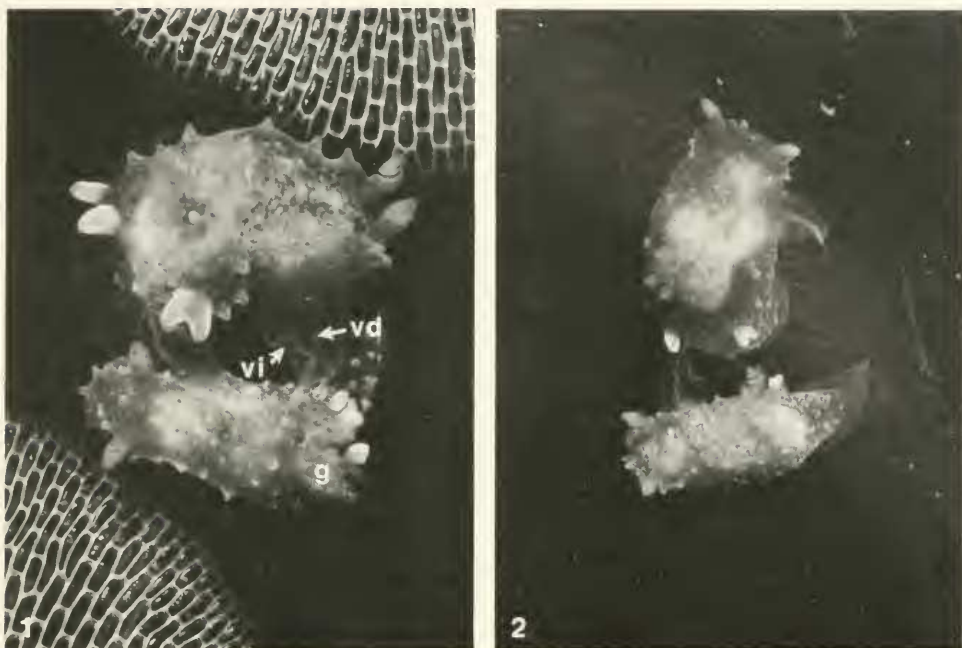


FIGURE 1. Two specimens of *Palio zosteræ* reciprocally copulating by hypodermic injection, with the penial cirrus of each penetrating the body wall below the gills (g) of the other. Within the expanded preputium of the upper animal the vas deferens (vd) and the blind vaginal in-pocketing (vi) can be seen. Each nudibranch is about 8–10 mm in length.

FIGURE 2. Non-reciprocal copulation in *Palio zosteræ*. Moments earlier these two nudibranchs had been copulating reciprocally. The penial cirrus has partially everted from the tip of the preputium of the upper nudibranch. Each animal is about 8–10 mm in length.

to serve at least three functions. First, when the preputium is pressed against the partner's body wall and the cirrus begins to evert, the barbs roll in a radiating fashion over the lip of the elongating cirrus, tearing a hole in the body wall. Second, the barbs then anchor the proximal region of the cirrus in the epidermal tissue as the cirrus continues to evert. Third, the barbs hold the penis in place even if the partner starts to move away. When this happens, the nudibranch may be dragged along by its cirrus. During bouts of copulatory activity, the cirrus may be everted when the preputium is not pressed against the partner (Fig. 2). Under these circumstances the cirrus has been measured to evert up to one mm beyond the tip of the preputium in an eight mm long *P. zosteræ*.

The site where the cirrus penetrates the partner's body wall varies, but since the majority of copulations are reciprocal, the point of penetration is usually in the posterior right flank of each nudibranch (Fig. 1). A cirrus piercing the body there is likely to penetrate the gonad, which lies a short distance beneath the body wall (Fig. 4). Hundreds of copulations have been observed, and never has the cirrus penetrated the vaginal in-pocketing, the preputium, or a site within the external genital opening. Of 88 copulations where the point of penetration was recorded, 53% of the penetrations occurred in the posterior right flank, 26% in the posterior left flank, 7% in the head, 3% in the foot, and 11% in other locations such as the rhinophores, dorsal surface, and gills. Reciprocal copulations last one to six minutes, with the cirrus often being

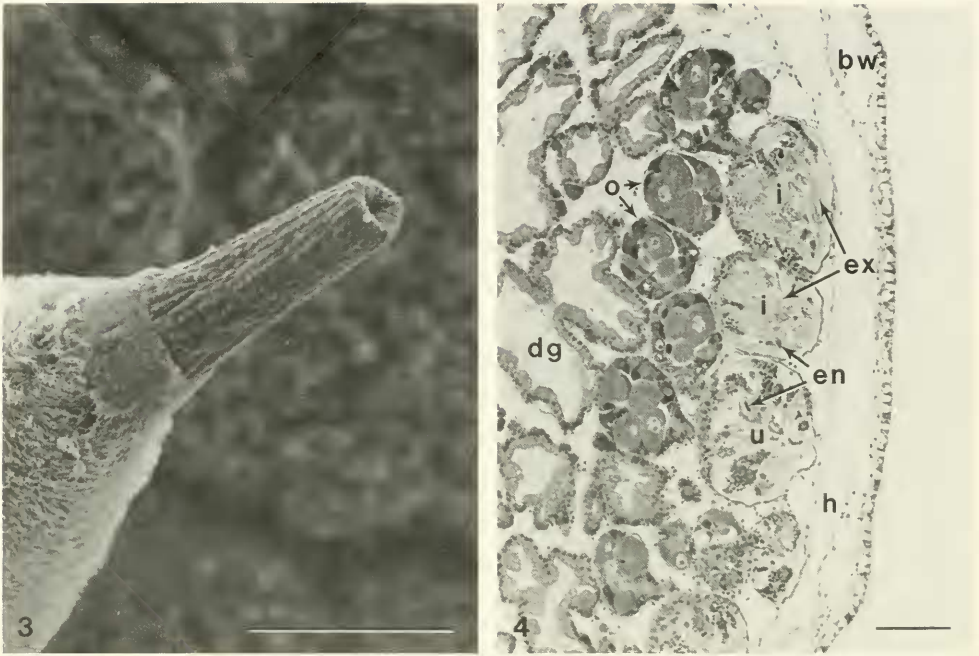


FIGURE 3. Scanning electron micrograph of a partially everted, barbed penial cirrus protruding from the tip of the ciliated preputium in *Palio zosteræ*. Bar = 100 μ m.

FIGURE 4. Paraffin section showing the organization of the visceral mass in *Palio zosteræ*. The relative position of the male and female acini is opposite that found in *Polycera*. This animal was fixed shortly after copulation, and the upper two spermatogenic acini visible here are filled with exogenous sperm. bw—body wall, dg—digestive gland, en—endogenous sperm or spermatids, ex—exogenous sperm, h—haemocoel, i—injected spermatogenic acinus, o—oogenic acini, u—uninjected spermatogenic acinus. Bar = 100 μ m.

retracted and reinserted at a new location several times during a copulatory bout. The cirrus is invaginated, disengaging its barbs, as it is withdrawn.

During copulation, peristaltic action of the muscular vas deferens moves sperm distally through the preputium into the cirrus. In some of the observed copulations, masses of sperm were deposited within the body wall and haemocoel of the partner. The sperm appeared as a white mass of various sizes up to 0.75 mm in diameter but usually much smaller. Some masses dissipated within 5–10 minutes; others remained visible through the body wall for up to six hours. Occasionally some sperm leaked out of the penetration wound or the tip of the cirrus as the penis was withdrawn from the partner.

Specimens of *Palio zosteræ* kept in a dish appeared to be attracted to copulating conspecifics and stimulated to participate in copulatory activity. Thus a pair of nudibranchs engaging in copulation were soon joined by one to several others, with individuals in the center of the group being injected simultaneously by up to four others. Groups of three copulating *P. zosteræ* were observed four times in the field, with the middle individual being injected in both the right and left sides.

The mature Atlantic *Palio dubia* is much longer than *P. zosteræ* (13–25 mm versus 7–11 mm). Although fewer observations were made on *P. dubia*, copulation appears no different than in *P. zosteræ*. During observed copulations in *P. dubia*, all

sides of the body were penetrated by the partner's cirrus, but the penetration site was usually in the posterior right flank during reciprocal copulation. Never was the cirrus inserted in the vaginal inpocketing or external gonopore.

Reproductive system anatomy in Palio

The reproductive systems of *Palio zosteræ* and *P. dubia* differ only in the relative sizes of some of the reproductive structures. Therefore, the following anatomical description of the reproductive system applies to both species.

The internal organs of *Palio* consist of three large masses: the buccal mass containing the jaws and radula at the anterior end of the animal, the anterior (or distal) reproductive organs just inside the genital pore, and the posterior visceral mass beneath the gills. These masses fill much of the large body cavity, but large haemocoelic spaces remain between them. The reproductive system of *Palio* is illustrated in Figure 5.

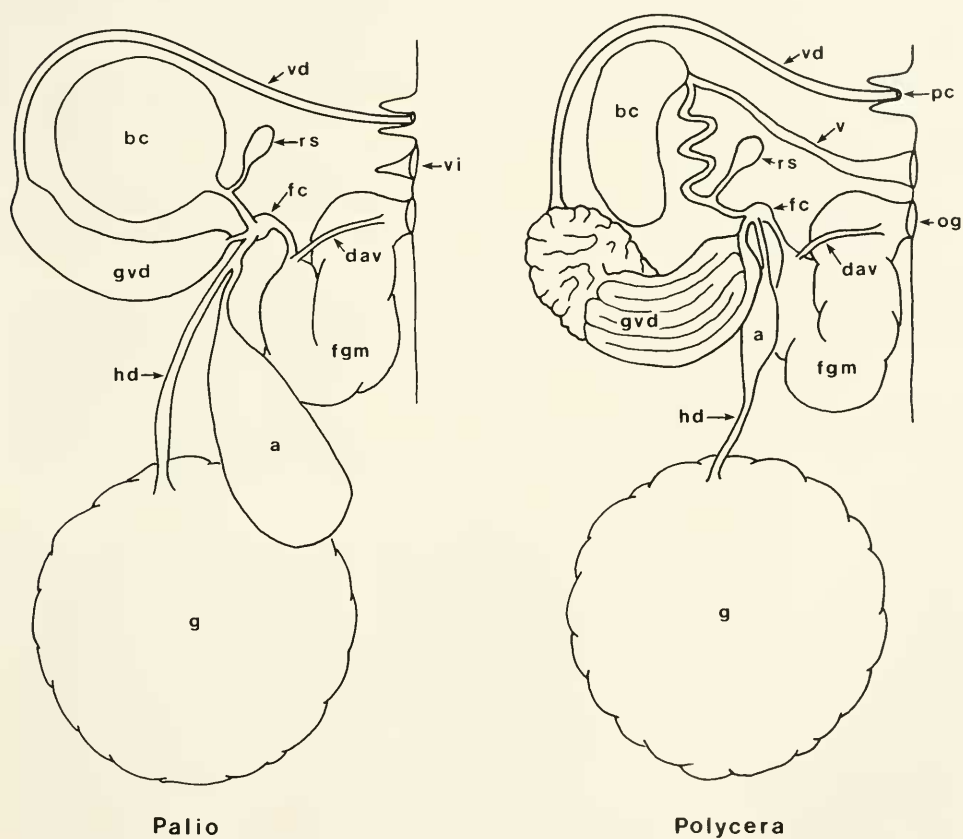


FIGURE 5. Schematic diagram of the reproductive systems of *Palio* and *Polycera*, based on observations on *Palio zosteræ*, *P. dubia*, *Polycera quadrilineata*, *P. faeroensis*, and *P. tricolor*. Dorsal view. The reproductive openings would normally be housed within an external common gonopore. a—ampulla, bc—bursa copulatrix, d—ductus-albumino-vestibularis, fc—fertilization chamber, fgm—female gland mass, g—visceral mass containing the gonad, gvd—glandular vas deferens, hd—hermaphroditic duct, og—oviducal gonopore, pc—partially everted penial cirrus, rs—receptaculum seminis, v—vaginal duct, vd—muscular vas deferens, vi—vaginal inpocketing.

The spherical visceral mass consists of a central stomach and digestive gland covered by the ovotestis. The ovotestis is organized into oogenic and spermatogenic acini. Several oogenic acini are connected via short necks to each spermatogenic acinus. Whereas there is little unoccupied space in the center of the oogenic acini due to the several developing ova bulging inward, the spermatogenic acini have a large lumen occupied peripherally by spermatogonia and spermatocytes with some maturing spermatids suspended towards the center. The relative position of these male and female acini in *Palio* is opposite that found in *Tritonia hombergi* (Thompson, 1961) and *Polycera* (pers. obs.), with the spermatogenic acini lying peripherally, instead of centrally. These spermatogenic acini lie just underneath the tissue covering the visceral mass. Beneath the spermatogenic acini are most of the oogenic acini (Fig. 4). A few oogenic acini are found at the surface of the visceral mass, but not on the right lateral surface where penetration by a cirrus is most likely to occur. A branch of the hermaphroditic duct arises from each spermatogenic acinus. This branch joins others to form a common hermaphroditic duct that leaves the anterior margin of the visceral mass and descends into the center of the anterior reproductive organs. Because of the gonadal arrangement, ova released from oogenic acini must pass through the spermatogenic acinus before reaching the hermaphroditic duct.

Near the middle of the anterior reproductive organ mass, the hermaphroditic duct gives off a short duct that reflects posteriorly and enlarges into a lacrimate ampulla (Fig. 4) in which endogenous sperm are presumably stored (Beeman, 1970a, b; Ghiselin, 1966). The ampulla measures about 1.5 mm in diameter and 3.5 mm long in a 10 mm long *Palio zosterae*. The white sperm mass in such a large ampulla is visible through the body wall. The ampulla was never empty, but was swollen with sperm even after an animal had copulated repeatedly.

Shortly after the ampullar duct is given off, the hermaphroditic duct splits into the vas deferens and the proximal oviduct. The vas deferens becomes enlarged, glandular, and somewhat flattened as it wraps around the posterior and medial sides of the bursa copulatrix. The columnar, ciliated prostatic gland cells of this region produce eosinophilic secretions that can be seen in the lumen of the duct in sections of animals that were sexually active when fixed. This prostatic region of the vas deferens attenuates into a muscular duct which may coil several times before it reaches the external gonopore. When not in use, the barbed cirrus is housed inverted within the distal region of the vas deferens.

Beyond the bifurcation of the hermaphroditic duct into the vas deferens and proximal oviduct, the proximal oviduct immediately enlarges into a fertilization chamber, the interior walls of which are ciliated and thrown into numerous folds. In some specimens the proximal oviduct is essentially non-existent, with the vas deferens splitting off from the fertilization chamber near the point where the hermaphroditic duct enters. Two other ducts join the fertilization chamber. One leads medially to the bursa copulatrix and receptaculum seminis. The second duct, the distal oviduct, reflects posteriorly along the descending hermaphroditic duct and expands into the albumen gland which leads to the capsule gland of the female gland mass. This then turns anteriorly into the large mucous gland leading to the oviducal gonopore. A short, tiny (25 μ m in diameter), ciliated duct bypasses the female gland mass by connecting the proximal end of the female gland mass directly to the distal end. This is the ductus-albumino-vestibularis first described in *Polycera quadrilineata* by Pohl (1905).

The spherical bursa copulatrix, presumably the initial storage site for exogenous sperm (Ghiselin, 1966) or possibly a gametolytic organ (Thompson and Bebbington, 1969; Beeman, 1970b), occupies much of the medial portion of the anterior repro-

ductive organ mass. It may be 0.7 mm in diameter in a *P. zosteræ* 7 mm long. The epithelial cells of the bursa copulatrix appear cuboidal and slightly glandular, and are ciliated near the opening to the duct. In virgin or recently mated animals, the bursa copulatrix contained a few sperm and some unidentifiable material. A few hours after copulations where sperm were injected into the gonad, the bursa copulatrix was packed with sperm.

A lacrimate receptaculum seminis, a storage organ for exogenous sperm (Beeman, 1970b), is at the end of a short duct that joins the distal end of the duct to the bursa copulatrix. In contrast to the ampulla and bursa copulatrix, the receptaculum seminis is a small structure only about 0.15 mm long and 0.06 mm in diameter in *P. zosteræ*. It may contain loosely or densely packed sperm, the heads of which are not embedded in or aligned with the sparsely ciliated epithelial lining as they are in other opisthobranch species (e.g., Ghiselin, 1966; Thompson, 1966; Beeman, 1970a, b).

The vaginal inpocketing visible in the expanded preputium of living animals can be followed in the serial sections. This inpocketing of non-glandular cells ends blindly, with no trace of a connection or vestigial structure continuing internally. Also, no duct or remnant of the duct connecting the bursa copulatrix or receptaculum seminis or their ducts to the gonopore exists. Only the vas deferens and the distal oviduct connect the reproductive organs to the outside.

Fate of injected sperm

Observations of sperm being injected into the haemocoel stimulated an initial hypothesis that exogenous sperm enter the reproductive system of the recipient through an opening, such as a ciliated coelomostome, which collected the sperm from the haemocoel and moved them into an appropriate duct of the reproductive system. Three observations argue against this hypothesis. First, no such opening was seen in sectioned material, although the possibility of a transient opening exists. Second, blood cells aggregate around clusters of sperm in the haemocoel, interstices of the ovotestis or in the body wall (Fig. 6). These blood cells appeared to be phagocytosing the wayward sperm; sperm heads were seen within some blood cells. Third, numerous examples of acini injected with exogenous sperm were found.

In order for a cirrus to inject sperm into a partner's ovotestis, it must penetrate the body wall, traverse part of the haemocoel, penetrate the tissue covering of the visceral mass, and puncture an acinus. This represents a minimum distance of 100 μ m in a 7 mm long *P. zosteræ*. In serial sections of animals fixed shortly after copulation, wounds in the body wall and tissue covering the visceral mass were found next to punctured spermatogenic acini filled with sperm (Fig. 7). In some animals, sperm were found near the path of penetration in the body wall, haemocoel, and spaces around the ovotestis. Injected spermatogenic acini can be distinguished from non-injected ones by the number and organization of the spermatozoa they contain. Uninjected acini have no or few unattached spermatozoa, but have maturing spermatids suspended in arrays in the central lumen. Much empty space surrounds these arrays. In contrast, injected acini are packed with loose spermatozoa (Figs. 5, 7). Some copulations result in such volumes of sperm being injected into a spermatogenic acinus that some sperm are forced through the hermaphroditic duct into adjacent male acini. Sperm were also found in the common hermaphroditic duct and in the fertilization chamber in some animals with a recently injected gonad.

Prostatic secretions are injected along with sperm during copulation in *Palio*. In serially sectioned specimens fixed within eight hours of copulation, the eosinophilic prostatic secretions were found in the body wall, haemocoel, interstices of the visceral

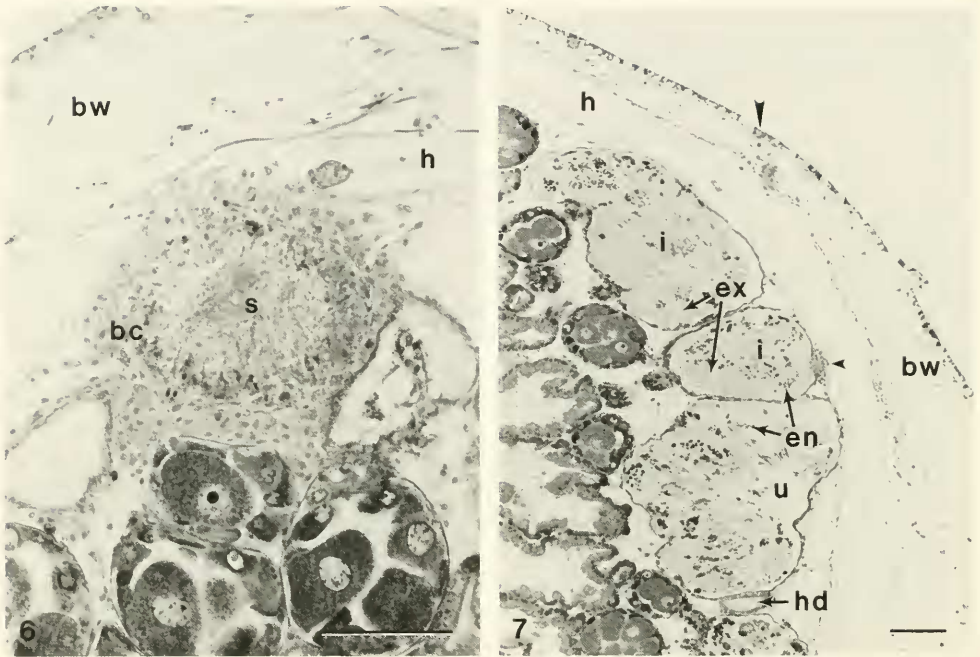


FIGURE 6. Sperm not injected into the gonad in *Palio* are phagocytosed by blood cells. This paraffin section of *P. zosteriae* shows a mass of sperm that was injected into a peripheral interstice of the ovotestis and is now surrounded by blood cells. bc—blood cells, bw—body wall, h—haemocoel, s—sperm. Bar = 100 μ m.

FIGURE 7. Paraffin section through the visceral mass of *Palio zosteriae* showing the pathway by which a penial cirrus penetrated the body wall (large arrowhead) and gonad (small arrowhead). This section shows two lobes of the same injected spermatogenic acinus filled with unoriented exogenous sperm. The broken, outwardly displaced section of the epidermis is an artifact. bw—body wall, en—endogenous sperm or spermatids, ex—exogenous sperm, h—haemocoel, hd—branch of the hermaphroditic duct, i—injected spermatogenic acinus, u—uninjected spermatogenic acinus. Bar = 100 μ m.

mass, and ovotestis. Sperm were mixed in with the prostatic secretions in animals that were fixed immediately after copulation. In other animals, injected secretions not within the gonad were without sperm, and were found concentrated in body spaces as if they had settled there due to gravity or due to the circulation of the blood. Prostatic secretions injected into the ovotestis appeared to be rapidly transported to the bursa copulatrix. Animals often had exogenous sperm still within spermatogenic acini and the hermaphroditic duct when the only hint of prostatic secretions within the reproductive system was eosinophilic globules within the bursa copulatrix.

The eight specimens of *P. zosteriae* reared to maturity in isolation never laid egg masses while isolated. When placed together, they copulated by hypodermic injection as described above. Most of these animals were fixed shortly after copulation, but the two animals that were kept together for two days each laid a fertile egg mass. They had copulated repeatedly by hypodermic injection, but did not appear to copulate in a manner considered normal in other nudibranchs in which the penis enters a vaginal opening.

Serial sections of the two specimens of *Palio zosteriae* reared in isolation but never allowed to copulate revealed in each an ampulla swollen with sperm, a bursa copulatrix

containing only a few sperm and unidentifiable debris, and an empty receptaculum seminis. In isolation animals that were fixed after being permitted to copulate, large numbers of unoriented sperm were seen in punctured spermatogenic acini. Sperm were found in the receptaculum seminis within four hours of copulation. In all of the mated isolation animals some of the injections hit the gonad while others missed.

Reproductive anatomy of Polycera spp.

To compare the reproductive systems of *Palio zosteræ* and *P. dubia* with that of other polycerid nudibranchs, specimens of *Polycera quadralineata*, *P. tricolor*, and *P. faeroensis* were serially sectioned and dissected. Aspects of the reproductive anatomy of *P. quadralineata* have been described by Pohl (1905), of *P. tricolor* by Robilliard (1971), and of *P. faeroensis* by Odhner (1941). The reproductive system of these three species of *Polycera* is illustrated in Figure 5. It is fundamentally organized like that in *Palio* except for four major differences:

First, the oogenic acini are peripheral to the spermatogenic acini, like that previously described for *Tritonia hombergi* (Thompson, 1961).

Second, the ampulla is not a separate sac as in *Palio*, but is just an enlarged region of the hermaphroditic duct. Also, in the three species of *Polycera* that were serially sectioned and dissected, the ampulla was relatively much smaller than in *Palio*.

Third, the prostate is more complex in *Polycera* than in *Palio* where it is simply an enlarged glandular region of the proximal vas deferens. In *Polycera*, the prostate has two distinct glandular regions. In the first glandular region the vas deferens is folded back on itself several times. The second region occurs immediately after the first, but consists of an area of highly convoluted walls lined by cells with different staining characteristics than those of the first region.

Fourth, a complete vaginal duct connects the vaginal opening in the gonopore complex with the bursa copulatrix, joining the bursa at the site where the duct to the fertilization chamber enters. The vaginal duct lies posterior to the cirrus and anterior to the oviducal pore, a position analogous to that of the vaginal inpocketing in *Palio*.

DISCUSSION

The taxonomic position of *Palio* (= *Polycera*) *zosteræ* needs clarification. The genus *Palio* was separated from the genus *Polycera* by Gray (1857), with *Palio* possessing a tuberculate, as opposed to a digitate, frontal margin. Although Eliot (1910), Lemche (1929), and Odhner (1941) suggested that *Palio* should not remain separate from *Polycera* due to the variable nature of external differences, *Palio* has remained in use (e.g., Swennen, 1961; Thompson and Brown, 1976). O'Donoghue described the Pacific nudibranch I have investigated as *Polycera zosteræ* in 1924, but based on its external morphology it belongs in Gray's genus *Palio*. The evidence provided above suggests that differences in the reproductive system can also be used to separate members of the genus *Palio* from *Polycera*: *Palio* lacks a vaginal duct, possesses an ampullar sac, and has spermatogenic acini peripherally located in the ovotestis. In this context, an examination of the reproductive systems of *Palio pallida* Bergh, 1880, *P. parvula* Burn, 1958, and *P. amakusana* Baba, 1960, would be useful.

Copulation by hypodermic injection in *Palio zosteræ* and *P. dubia* contrasts strikingly to the reciprocal intromission of a penis into a vaginal duct found in other nudibranchs (Costello, 1938; Haefelfinger, 1960; Rutowski, 1983). In *Palio*, the cirrus

penetrates the body wall to inject sperm either into the gonad, or into the haemocoel where the sperm are phagocytosed. The phagocytosed sperm may provide some nourishment, albeit small, to the recipient. In some non-molluscan species, material donated during mating contributes to the energy and material needs of the recipient (Hinton, 1964; Boggs and Gilbert, 1979).

Whereas copulation by hypodermic injection has not been previously reported in nudibranchs, it has for some ascoglossan opisthobranchs. The ascoglossans *Limapontia cocksi* and *L. capitata* (Gascoigne, 1956) and *Stiliger fuscatus* (Gascoigne, 1978) have a pseudodiaulic reproductive system. No vaginal opening exists but reciprocal copulation occurs with a penial stylet piercing the body wall and depositing sperm in the underlying bursa copulatrix. Alignment of the copulating animals in these species is essential for the bursa to be pierced. In two other ascoglossan species, *Elysia maoria* (Reid, 1964) and *Alderia modesta* (Hand and Steinberg, 1955; pers. obs.), copulation is also by hypodermic injection, but there is no preferred point of injection where an internal organ would be consistently pierced. The fate of injected sperm in these species is unknown.

Assuming that exogenous sperm fertilize eggs, the mode of copulation in *Palio* has a risk that sperm will not be injected into the partner's gonad and hence be wasted. However, this risk is minimized by the animals' behavior. Reciprocal copulation in *Palio* is not requisite but is the norm. During reciprocal copulation the cirrus penetrates the right flank where it is likely to hit the ovotestis.

The selective advantages of the mode of copulation found in *Palio* is enigmatic. *Palio* has lost the complete vaginal duct found in other nudibranchs. Regression in structure and function in the reproductive system is found in some interstitial molluscs, presumably because of space limitations (Swedmark, 1968). Such a space constraint is unlikely in *Palio zosterarum*, which is larger than many other species of normally copulating nudibranchs. From an energetic standpoint, the mode of copulation exhibited by *Palio* may be more expensive than the normal mode because of misaimed injections. *Palio* has a relatively large ampulla compared with that in the normally copulating *Polycera*, suggesting that more sperm are needed per successful copulation.

Exogenous sperm are injected into the spermatogenic acini of *Palio* where endogenous sperm are made. With the techniques utilized in this study, endogenous sperm could not be distinguished from exogenous sperm in places other than recently injected acini. Therefore, what happens to sperm injected into the gonad can only be hypothesized based on the observations of individuals reared in isolation along with assumptions of functional anatomy of the reproductive system based on information from the literature (e.g., Ghiselin, 1966; Beeman, 1970a, b). Both endogenous and exogenous sperm travel down the hermaphroditic duct to the anterior reproductive organs. Endogenous sperm are passed into the ampulla while exogenous sperm travel through the fertilization chamber to the receptaculum seminis. The reproductive system in *Palio* thus may have endogenous and exogenous sperm coexisting in the gonad and hermaphroditic duct. How the reproductive system distinguishes exogenous from endogenous sperm in its sorting process is unknown. One possible difference between the two types of sperm is that the exogenous sperm may have been capacitated by secretions from the donor's prostate (Thompson, 1966). But if sperm capacitation due to prostatic secretions results in character differences used for sperm sorting, and if the action of prostatic secretions is not self-specific, then a problem arises. Since prostatic secretions are injected along with sperm during copulation, endogenous sperm might be capacitated, stored in the receptaculum seminis, and used for fertilizing eggs, resulting in self-fertilization. However, the chances of fertilization by endogenous sperm capacitated by exogenous prostatic secretions is low because few mature en-

ogenous sperm are in the spermatogenic acini or hermaphroditic duct at any given time. Therefore, few mature endogenous sperm would be exposed to injected prostatic secretions.

The movement of *Palio zosteræ* to nearby copulating conspecifics and their subsequent involvement in the copulatory activity suggest a chemically mediated attraction. Chemical signals apparently stimulate aggregation and breeding activity in *Aplysia*, an anaspidian opisthobranch which mates in long chains (Audesirk, 1977; W. Aspey, pers. commun.). Nudibranchs normally mate in pairs and not in chains (Costello, 1938; Hyman, 1967), but *Palio* is unlike other nudibranchs. The mode of copulation in *Palio* permits more than two individuals to participate simultaneously. By attracting others, mating individuals may increase the chance that they will be successfully inseminated.

Presumably *Palio* evolved from a polycerid ancestor possessing a triaulic reproductive system. The layout of the reproductive system in *Palio* is like that in *Polycera* except for the four differences mentioned above. The vaginal inpocketing in *Palio* has no apparent function, but is in the same relative position as the vaginal opening in *Polycera*. The inpocketing is probably a remnant of an ancestral vaginal opening. The loss of the vaginal duct might have been the first alteration in the ancestral triaulic reproductive system, and other modifications arose as a consequence of the new mode of copulation. The barbed cirrus used for penetrating the body wall during copulation in *Palio* is also a characteristic of members of the genus *Polycera* (Bergh, 1880; Odhner, 1941; Pruvot-Fol, 1954; Marcus and Marcus, 1967; Robilliard, 1971). An armed, evaginating cirrus is probably ancestral in *Palio*, and was therefore preadapted for use as a penetrating organ during copulation by hypodermic injection.

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